PRODUCTION OF BETA-CAROTENE BY USING *DUNALIELLA SALINA*: EFFECT OF $NaNO_3$ CONCENTRATION AND LIGHT INTENSITY

NUR ISMA IDAYU BINTI ISMAIL

A thesis submitted in fulfillment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JUNE 2012

ABSTRACT

Beta-carotene is used as vitamin A precursor and its antioxidant property has been used in dietary supplements for the prevention of diseases such as cancer and coronary heart disease. The purpose of this study is to study the effect of $NaNO_3$ concentration and light intensity to the growth and production of beta-carotene by *Dunaliella salina*. Four different $NaNO_3$ concentrations (100%, 75%, 50%, and 25% v/w) and light intensities (6000 lx, 8000 lx, 11000 lx and 17000 lx) were used. Cell growth was determined by cell counting method and analysis of total beta-carotene concentration in the samples was done using High Performance Liquid Chromatography (HPLC). From the result obtained, the $NaNO_3$ concentration and light intensity will affect the growth of *Dunaliella salina,* where the growth was reduced due to $NaNO_3$ starvation and it was also induced with the high light intensity until nitrate became the limiting source for the cells. Besides that, $NaNO_3$ concentration and light intensity will also affect the beta-carotene production of *Dunaliella salina,* where the production was increased as the $NaNO_3$ concentration was decreased and the light intensity was increased until the concentration of $NaNO_3$ has become the limiting factor of the experiment. In order to improve the research, further study to identify the optimal $NaNO_3$ concentration and light intensity required for maximizing the production of beta-carotene by *Dunaliella salina* shall be done.

ABSTRAK

Beta-karotena digunakan sebagai pelopor vitamin A dan sifat antioksidan telah digunakan dalam makanan tambahan pemakanan untuk mencegah penyakit seperti kanser dan penyakit jantung koronari. Tujuan kajian ini adalah untuk mengkaji kesan kepekatan $NaNO_3$ dan keamatan cahaya kepada pertumbuhan dan pengeluaran beta-karotena oleh *Dunaliella salina*. Empat $NaNO_3$ dengan kepekatan yang berbeza (100%, 75%, 50%, dan 25% v / w) dan keamatan cahaya (6000 lx, 8000 lx, 11000 lx dan 17000 lx) akan digunakan untuk mengkaji kesan parameter pada pertumbuhan dan pengeluaran beta-karotena oleh *Dunaliella salina*. Pertumbuhan sel ditentukan oleh kaedah pengiraan sel dan analisis kepekatan jumlah beta-karotena dalam sampel telah dilakukan dengan menggunakan Kromatografi Cecair Prestasi Tinggi (HPLC). Berdasarkan keputusan yang diperolehi, kepekatan $NaNO_3$ dan keamatan cahaya akan menjejaskan pertumbuhan *Dunaliella salina* di mana pertumbuhan telah dikurangkan disebabkan oleh kekurangan $NaNO_3$ dan ia juga disebabkan dengan keamatan cahaya yang tinggi sehinggalah nitrat menjadi sumber terhad untuk sel-sel. Selain itu, kepekatan $NaNO_3$ dan keamatan cahaya juga akan menjejaskan pengeluaran beta-karotena oleh *Dunaliella salina* di mana pengeluaran telah meningkat apabila kepekatan $NaNO_3$ telah menurun dan keamatan cahaya meningkat sehinggalah kepekatan $NaNO_3$ telah menjadi faktor penghad eksperimen. Dalam usaha untuk meningkatkan penyelidikan, kajian lanjut perlu dilakukan untuk mengenalpasti kepekatan $NaNO_3$ dan keamatan cahaya yang optimum diperlukan untuk memaksimumkan pengeluaran beta-karotena oleh *Dunaliella salina*.

TABLE OF CONTENT

		Page
SUPERVISOR'S DECLARATION		ii
STUDENT'S DECLARATION		iii
DEDICATION		iv
ACKNOLEDGEMENTS		v
ABSTRACT		vi
ABSTRAK		vii
TABLE OF CONTENTS		viii
LIST OF TABLES		x
LIST OF FIGURES		xi
LIST OF SYMBOLS		xii
LIST OF ABBREVIATIONS		xiv
CHAPTER 1	**INTRODUCTION**	
1.1	Background of Study	1
1.2	Problem Statement	2
1.3	Objective	3
1.4	Scopes of research	4
1.5	Rational and Significance	4
CHAPTER 2	**LITERATURE REVIEW**	
2.1	Commercial green microalgae	5
2.2	Mode culture of *Dunaliella salina*	7
2.3	*Dunaliella salina*	8
2.4	Halotolerance	10

2.5	Carotenoids	11
2.6	Beta-carotene	13
2.7	Effect of nitrogen starvation on growth and production	14
2.8	Effect of light intensity on growth and production	15

CHAPTER 3 METHODOLOGY

3.1	Culture of microalgae	17
3.2	Preparation for different $NaNO_3$ concentration	19
3.3	Effect of different light intensity	19
3.4	Growth measurement	19
3.5	Microalgae harvesting and extraction of carotenoids	21
3.6	Identification of beta-carotene	21

CHAPTER 4 RESULT AND DISCUSSION

4.1	Effect of $NaNO_3$ concentration and light intensity to the growth of *Dunaliella salina*	22
4.2	Effect of $NaNO_3$ concentration and light intensity to the total beta-carotene production in *Dunaliella salina*	24

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

5.1	Conclusion	28
5.2	Recommendations	28

REFERENCES 29

APPENDICE

A	Soil extract recipe	34
B1	Average cell density (cells/ml) of different $NaNO_3$ concentrations and light intensities	35
B2	Beta-carotene production of different $NaNO_3$ concentration and light intensity	36

LIST OF TABLES

Table No.	Title	Page
3.1	Stock solution recipe for 2ASW medium	18
3.2	Recipe for 2ASW complete medium	18
B1	Average cell density (cells/ml) of different $NaNO_3$ concentrations and light intensities at 7 days	35
B2	Beta-carotene (mg/ml) production at different $NaNO_3$ concentration and light intensity	36

LIST OF FIGURES

Figure No.	Title	Page
2.1	*Dunaliella salina* cells in different culture condition.	10
2.2	Health promoting functions attributed to carotenoids.	11
2.3	Structures of the biotechnologically most important carotenoids.	12
2.4	Chemical structure of beta-carotene isomer.	14
3.1	Hemocytometer	20
3.2	Hemocytometer counting chamber	20
4.1	Effect of light intensity and $NaNO_3$ concentration on the growth of *Dunaliella salina*	23
4.2	Effect of light intensity and $NaNO_3$ concentration on beta-carotene production	26

LIST OF SYMBOLS

^{o}C	Celsius
cm^3	Cubic centimeter
g	Gram
g/l	Gram per liter
m	Meter
m^2	Square meter
$m^2.s^{-1}$	Square meter per second
mg/ml	Milligram per milliliter
min	Minutes
ml	Milliliter
ml^{-1}	Per milliliter
$ml.min^{-1}$	Milliliter per minutes
mm	Millimeter
mm^2	Square millimeter
$m.s^{-1}$	Meter per second
nm	Nanometer
tonnes/year	Tonnes per year
US$/year	United State Dollar per year
W	Watt
w/v	Weight per volume
v/v	Volume per volume
β	Beta

µm	Micrometer
µmol.$m^{-2}s^{-1}$	Micromole per square meter second
%	Percent

LIST OF ABBREVIATIONS

ASW	Artificial Seawater
C	Carbon
CCAP	Culture Collection of Algae and Protozoa
CO_2	Carbon dioxide
O_2	Oxygen
HCl	Hydrochloric acid
HPLC	High Performance LiquidChromatography
K_2HPO_4	Dipotassium phosphate
KNO_3	Potassium nitrate
N	Nitrogen
NaCl	Sodium chloride
$NaNO_3$	Sodium nitrate
NO_3^-	Nitrate
NH_4^+	Ammonium

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

There are more than 25,000 species of microalgae which only 15 are in use. The technology in culturing microalgae becomes business oriented owing to their practical applications. Beta-carotene production by using green microalgae *Dunaliella* is a major success in applied algae biotechnology. Halotolerant algae of genus *Dunaliella* is a eukaryotic microorganism that can survive in a very high salinity condition. This microalga has the ability to adapt to sudden change in salinity, irradiance, nutrient availability, and light intensity (Lamers et al., 2008). According to Marín et al. (1998), recent researches on potential therapeutic properties of beta-carotene have also contributed to the interest in this microalga. Beta-carotene preparations have been shown to inhibit or prevent various types of tumors in humans and animals, including skin cancers such as melanoma, epidermoid cancers of head and neck, cancers of the gastrointestinal tract, carcinomas of secretory glands such as the pancreas and breast cancer (Tafreshi and Shariati, 2009). Besides that, beta-carotene is also effective in controlling cholesterol levels and reducing the risk of cardiovascular diseases, such as myocardial infarction and angina pectoris as well as coronary heart disease.

One type of this microalgae species, *Dunaliella salina* is known to store a large capacity of beta-carotene and glycerol. Beta-carotene content in *Dunaliella salina* may reach until 10% of its cell dry weight (Lamers et al., 2008; Zamani et al., 2011). This product is directed mainly at the natural food colorant, nutraceutical or health food market (Barzegari et al., 2010; Celekli and Donmez, 2006). *Dunaliella salina* is a suitable source for commercial production of beta-carotene and preferentially accumulates 9-cis-isomer which functions as a carbon sink. According to Raja et al. (2008), the natural 9-cis isomer plays a major role in quenching free oxygen radicals and preventing oxidative damage to the cell. The free radicals are highly reactive molecules and they can cause harmful reactions for example uncontrolled oxidation in the body damages fat, genetic materials, and cell membranes including cataracts and cardiovascular diseases. The 9-cis isomer is synthesized only by natural sources and it is a highly valuable metabolite because of its interesting physicochemical high liposolubility properties, which makes it being effectively preserved into animal tissues; furthermore, this isomer has proved to have a better antioxidant capacity than the all-trans isomer (Gómez et al, 2003).

There are many studies about the effect of salinity on the quantity and quality of beta-carotene production of *Dunaliella salina* (Hadi et al, 2008; Gómez et al, 2003; Pisal and Lele, 2005). Previous studies suggest that the stressful environmental conditions, like high irradiance and salinity, favor high production of carotenoids (Marin et al., 1998).

1.2 PROBLEM STATEMENT

Most of beta-carotene supplements in the market are synthetic which consisted of only all-trans isomer. Tafreshi and Shariati (2009) stated that, several studies have reported that the synthetic all-trans beta-carotene not only fails to reduce the incidence of cancer, but it may be carcinogenic. But in natural beta-carotene found in plants like carrot, avocado, broccoli, coffee, watermelon and microalgae like *Dunaliella salina*, it contains both all-trans beta-carotene and 9-cis beta-carotene. The 9-cis isomers in natural beta-carotene supplement is used as antioxidant since they have greater antioxidant effect and therapeutic effect in the

prevention of diseases such as cancer and coronary heart disease than the all-trans isomer (Levin and Mokady, 1994).

The commercial production of beta-carotene by *Dunaliella salina* has to face the dilemma that its production is enhanced by physiological stress (Sánchez-Estudillo et al., 2006). Giordano and Beardall (2009) stated that, beta-carotene accumulation is highest when the growth is lowest. But low growth of these microalgae will produce low biomass production. Under stress conditions such as nutrient limitation, high salinity and high light intensity, *Dunaliella salina* reacts by accumulating a large amount of beta-carotene as its secondary metabolite instead of producing chlorophyll as its primary metabolite.

Several strategies have been used to maximize the production of beta-carotene per unit time and per culture volume. These strategies are based on the observations that severe conditions, such as high salinities, low nutrient levels and high temperatures combined with high irradiance, retard growth and at the same time, induce beta-carotene production in the cell. The higher the stress intensity and as a result the slower the growth rate of the alga, the greater is the total amount of the light absorbed by the cell during one division cycle. This situation can lead to higher accumulation of beta-carotene per cell. However, these conditions at the same time decrease the cell number per unit culture volume by affecting cell viability. Moreover, prolonged nutrient starvation can lead to high mortality of the algae. Therefore, it is recommended by one group of authors that adjusting light and salinity likely is one of the best strategies to achieve optimal beta-carotene production in mass cultures of *Dunaliella salina* (Tafreshi and Shariati, 2009).

1.3 OBJECTIVE

The objective of this study is to study the effect of $NaNO_3$ concentration and light intensity to the growth and production of beta-carotene by *Dunaliella salina*.

1.4 SCOPE OF RESEARCH

The scopes of the research are as follows:

a) Study the effect of different $NaNO_3$ concentration and light intensity to the growth of *Dunaliella salina*.
b) Study the effect of $NaNO_3$ concentration and light intensity in the production of beta-carotene by *Dunaliella salina*.

1.5 RATIONALE AND SIGNIFICANT

Carotenes in *Dunaliella salina* contain a mixture of alpha-carotene and beta-carotene isomers which consist of 9-cis and all-trans beta-carotene. This natural 9-cis isomer is rapidly used up in quenching free radicals and preventing oxidative damage to cell (Borowitzka, 1992). Furthermore, the pre-cancerous tissues in people are reverted to normal with the natural beta-carotene supplement but not with synthetic supplements. So, this research is important to produce natural beta-carotene which contains 9-cis and all-trans isomers for better antioxidant effect. Besides that, microalgae are used in the production of beta-carotene because of its fast growth rate, the ability of to grow over wide salinity range and the ease of manipulation (Barzegari et al., 2010; Borowitzka and Siva, 2007).

CHAPTER 2

LITERATURE REVIEW

2.1 COMMERCIAL GREEN MICROALGAE

According to Lamers et al. (2008), microalgae are photosynthetic microorganisms with which many added-value compounds can be produced in food, feed, cosmetics applications and feedstock for the chemical industry, and they also have potential as sustainable energy carriers. The microalgae produce high value molecules such as fatty acids, pigments and stable isotope biochemicals. For most of the applications, the market is still developing and the biotechnological uses of microalgae will extend to a new area (Raja et al., 2008).

Referring to Norton et al. (1996), it has been estimated that between 22,000 and 26,000 species exist, of which only several species have been identified to be useful for commercial application. These species are *Spirulina*, *Chlorella*, *Haematococcus*, *Dunaliella*, *Botryococcus*, *Phaeodactylum*, and *Porphyridium*. *Dunaliella salina* has probably been the most successful microalgae for the mass cultivation especially due to its high salinity requirement that minimizes the number of competitors and predators. Algal biotechnology has made major advances in the past three decades, and microalgae like *Dunaliella* are cultivated for the production of carotenoids and glycerol (Hadi et al., 2008). In this study, the focus will be discussing on the production of carotenoids in *Dunaliella salina*. The photosynthetic

ability is the most important feature of microalgae which makes them the promising organisms for autotrophic cultivation on simple mineral media for various biotechnological purposes.

According to Pulz and Scheibenbogen (1998), many attempts have been made to cultivate them in simple systems such as shallow open ponds since microalgae are very efficient solar energy converter. There are around 110 commercial producers of microalgae in the Asia-Pacific region, with an annual production capacity reach 500 tonnes. About nine-tenths of algal cultivation plants are located in Asia. This biomass of microalgae market has a size of about 5,000 tons/year of dry matter and generates a turnover of US\$1.25 × 10^9/year (Raja et al., 2008). In spite of such attractive feature of cultivating microalgae, several phototrophic single-species cultivation has met only limited success. Contamination by bacteria and protozoa has made such propagation possible only if suitable selective environments can be measured (Margalith, 1999). For examples, *Spirulina platensis* is successfully cultivated in high alkaline water which pH higher than 9.2 and *Dunaliella salina* is being cultivated commercially in open ponds with highly saline brine. The fast-growing microalgae such as *Chlorella* species can be also grown in open ponds and this system demand comparatively low investment for construction and maintenance. Economically feasible production of added-value compounds with microalgae is possible because microalgae produce biomass and specific biomass ingredients directly from solar irradiation at high photosynthetic efficiencies and high volumetric and a real productivities (Lamers et al., 2008). Brennan and Owende (2010) stated that under natural growth conditions, phototrophic microalgae absorb sunlight, and assimilate carbon dioxide from the air and nutrients from the aquatic habitats. According to Margalith (1999), microalgae which utilize organic carbon substrates as their sole carbon and energy source may be employed for heterotrophic growth. To prevent growth inhibition in the culture, usually very low concentrations of organic compounds are employed. This may be circumvented by using a suitable fed-batch system but heterotrophic media used may invite rapid bacterial contamination, which again may be overcome only by rigorous aseptic operations. The natural conditions for commercial algae production have the advantage of using sunlight as a free natural resource. However, it can be limited by available sunlight due to diurnal cycles and the seasonal variations (Brennan and

Owende, 2010). To address the limitations in natural growth conditions with sunlight, artificial lighting means employing fluorescent lamps are almost exclusively used for the cultivation of phototrophic algae at pilot scale stages. The artificial lighting allows for continuous production, but at significantly higher energy input.

2.2 MODE CULTURE OF *DUNALIELLA SALINA*

Algae cultivation in open pond production systems has been used since the 1950s which can be categorized into natural water (lakes, lagoon and ponds) and artificial ponds or containers (Brennan and Owende, 2010). According to Borowitzka et al. (1984), pilot plant site of *Dunaliella salina* at Hutt Lagoon is a shallow salt lake near Geraldton in Western Australia. The pilot plant facility consists of a number of open ponds with a total area of about 3000 m^2, constructed of earth walls set on the lake bed, and a shore compound housing the laboratory, workshop, water storage tanks and generator house. Water sources for the pilot plant are natural brines from the lake and fresh water from a well sunk on the shore. The current state of the corresponding production technologies are based on either open pond systems or closed photobioreactors (Campo et al., 2007). The potential of scientific and technological advances for improvements in yield and reduction in production costs for carotenoids from microalgae. Many types of system have been designed for the growth and handling of this microalgae on a large scale. Within the open pond modes, the best choice is the open shallow pond which made of leveled raceway 2-10 m wide and 15-30 cm deep and the running is as simple loop or meandering systems. Each unit covers an area of several hundred to a few thousand square meters. Turbulence is usually provided by rotating paddle wheels, which create a flow of the algal suspensions along the channels at a rate of 0.2–0.5 m s^{-1}. The adequate supply of carbon dioxide is very critical, and it is usually controlled through a pH-stat, so warranting both provision of carbon and optimum pH of the culture simultaneously.

Production of microalgae based on closed photobioreactor is designed to overcome some of the major problems associated in open pond system such as pollution and

contamination risk. However, compared with the cultivation of open pond system, this closed photobioreactor system cost is substantially higher. Tubular, flat plat and column photobioreactors are the examples of closed system. Brennan and Owende (2010) stated that this closed system is more appropriate for sensitive strains because it makes the control of potential contamination becomes easier and this system is usually used for the production of high-value pharmaceutical and cosmetic products. Photobioreactor consist of an array of straight glass or plastic tubes which aligned horizontally or vertically. The microalgae culture will be re-circulated either with a mechanical pump or air-lift system to allow CO_2 and O_2 exchange between the medium and aeration gas as well as to provide mixing.

2.3 *DUNALIELLA SALINA*

On 1938, Dunal had discovered the alga which gave the reddish colour of salt water during production of sea salt in southern France (Oren, 2005). According to Tafreshi and Shariati (2009), *Dunaliella* species belong to the phylum *Chlorophyta*, order *Volvocales* and family *Polyblepharidaceae*, and are unicellular, photosynthetic and motile biflagellate microalgae morphologically distinguished by the lack of a rigid cell wall. García et al. (2007) stated that, there are 23 species of the genus *Dunaliella* found in saline environments and exhibiting optimal growth at different salt concentrations with varying abilities to turn orange-red under particular culture conditions. The famous species of *Dunaliella* are *Dunaliella salina, Dunaliella tertiolecta, Dunaliella primolecta, Dunaliella viridis, Dunaliella bioculata, Dunaliella acidophyla, Dunaliella parva* and *Dunaliella media*. Borowitzka and Siva (2007) have assessed current issues of the taxonomy of *Dunaliella. Dunaliella* cells are found to be ovoid, spherical, pyriform, fusiform or ellipsoid with length from 5 to 25 µm and width from 3 to 13 µm (Oren, 2005; Ramos et al., 2011). These motile cells are also biflagellate with the flagella inserted at the anterior end of the cell the flagella length also varying between species. The cells also contain a single cup-shaped chloroplast which mostly has a central pyrenoid surrounded by starch granules. The genus of *Dunaliella* have been in the subject of numerous studies as a result of several factors such as the ease of culturing, the ability of several species to grow over wide salinity ranges and at extreme salinities, the accumulation of extremely

high levels of beta-carotene in *Dunaliella salina*, and lastly the wide tolerance to heavy metals and pesticides by some species. Besides chlorophylls a and b, the members of *Dunaliella* contain valuable carotenoid pigments such as alpha-carotene and beta-carotene, violaxanthin, neoxanthin, zeaxanthin and lutein (Ye et al., 2008).

Teodoresco was the first to describe *Dunaliella salina* in 1950 and named it after Dunal (Borowitzka and Siva, 2007) and this alga often found in natural marine habitats which make the water reddish in colour (Giordano and Beardall, 2009). The vegetative cell division of *Dunaliella salina* commences with the nuclear division followed by a furrowing of the cell at the anterior or flagella end of the cell and then at the opposite or posterior of the cell. The furrowing generally proceeds faster at the flagella end and the posterior furrowing proceeds with the division of the chloroplast and pyrenoid which then produce two daughter cells. The massive accumulation of beta-carotene by the strains under suitable growth conditions has led to interesting biotechnological applications and this pigment is primarily composed of the isomers 9-cis and all-trans (Gómez et al., 2003). The 9-cis beta-carotene occurs only in natural sources and is the most attractive from a commercial point of view. The result from the previous study showed that the 9-cis beta-carotene has higher antioxidant potency than that of the all-trans isomer (Levin and Mokady, 1994). According to Ye et al. (2008), *Dunaliella* have been exploited commercially to yield dried biomass and natural beta-carotene in several countries such as Israel, China, USA, Australia and Mexico since 1980s. The synthesis of beta-carotene increases with the unbalances physiological condition of cell due to stress factor (Pisal and Lele, 2005). This alga accumulates large amounts of beta-carotene as droplets in the chloroplast, to prevent chlorophyll photo-damage, when culture conditions include high light intensities, high temperature, high salinity and deficiency of nutrient (Lamers et al., 2008; Tafreshi and Shariati, 2009). The figure of *Dunaliella salina* in different culture condition was shown in **Figure 2.1**.

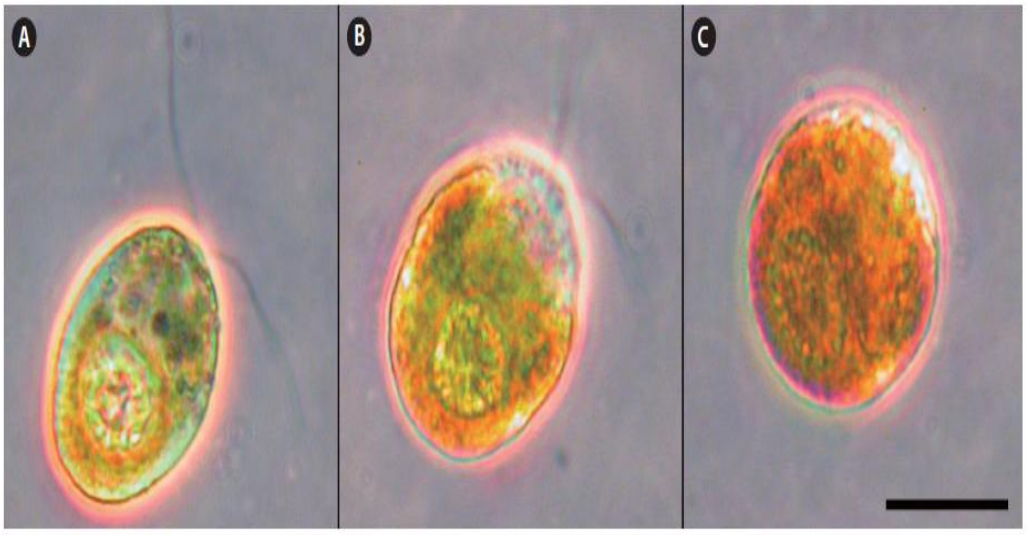

Figure 2.1: *Dunaliella salina* cells in different culture condition. (A) Green cell from a non-stressed culture. (B) Stressed cell turning orange. (C) Orange cell from a culture exposed to nutrient stress due to beta-carotene accumulation (Ramos et al., 2011)

2.4 HALOTOLERANCE

According to García et al. (2007), halotolerant algae of genus *Dunaliella* is microorganisms that live in a very saline environment and can even survive in saturated salt solution. These organisms have the ability to adapt to sudden changes in salinity, irradiances and nutrient depletion in their habitats. *Dunaliella salina* has the potential to overcome these difficulties and is widely employed for the production of valuable fine chemicals such as carotenes. Previously, many researchers have manipulated on the stress factors and alternative work systems for massive production of beta-carotene (Ye et al., 2008). Under suitable conditions, some strains of *Dunaliella* could accumulate 10% or more beta-carotene of the total dry organic matter in weight (Hejazi and Wijffels, 2003; Lamers et al., 2008). High light intensity leads to an increase in the ratio of 9-cis to all-trans isomer (Liu et al., 1996). Several physiological adaptations have been developed by *Dunaliella* including the lack of a rigid cell wall, a variable intracellular concentration of glycerol changes in photosynthetic pigments balance and structural modifications in the chloroplast.

2.5 CAROTENOIDS

Carotenoids (C_{40}) are a group of natural fat-soluble pigments which are found in plants, algae, and photosynthetic bacteria (Stahl and Sies, 2005). They are responsible for the color in some species of yeast, bacteria and fungi as well as many vegetables and fruits. According to Lamers et al. (2008), the colors of these pigments range from yellow to red and some found in tomatoes (lycopene), maize corn (zeaxanthin) and carrot (beta-carotene).They play an important role in photosynthesis and there are about 600 different compounds of carotenoid that have been identified so far, of which 50 can be found in the human diet (Tourniaire et al., 2009). Carotenoids represent one group of valuable molecules for several industries such as pharmaceutical, chemical, food and feed industries. It is not only because they can act as vitamin A precursors, but also for their coloring, antioxidant and possible tumor-inhibiting activity. (Frengova and Beshkova, 2009). **Figure 2.2** shows several health promoting functions attributed to carotenoids.

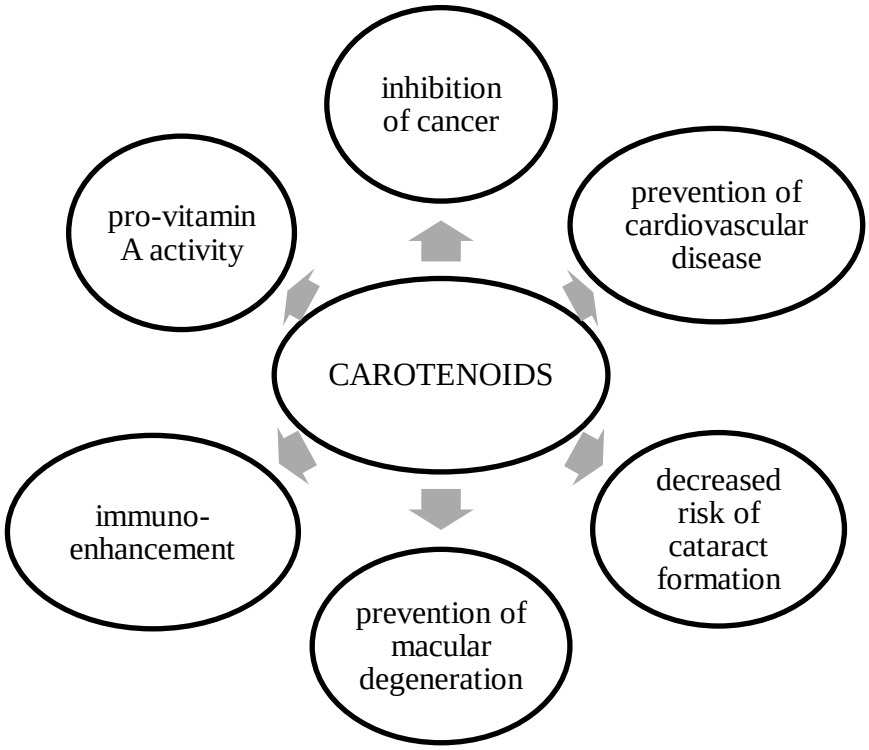

Figure 2.2: Health promoting functions attributed to carotenoids (Dutta et al., 2005)

According to Dutta et.al (2005), carotenoids are isoprenoids compounds which are biosynthesized by tail to tail linkage of two C_{20} geranylgeranyl diphosphate molecules and produce the parent C_{40} carbon skeleton from which all individual variation are derived. The skeleton can be modified by three factors which are cyclization at one end or both ends of the molecule to give different end groups, changes in hydrogenation levels and the addition of oxygen containing functional groups. They are separated into two groups where the first group contains hydrocarbons such as alpha-carotene and beta-carotene, the second group consists the oxygenated carotenoids such as lutein, zeaxanthin and astaxanthin (Ginka and Dora, 2009). The structure of these carotenoids found in *Dunaliella* was shown in **Figure 2.3**. According to Kleinegris et al. (2010), carotenoids protect the cell from damage by light and oxygen. It functions as accessory pigment in light harvesting, but in addition, they are important for protecting photosynthetic organisms from destructive photooxidation which can occur in the presence of light, oxygen and chlorophylls (Campo et al.,2007; Lamers et al., 2008). In plants the presence of carotenoids is often masked by chlorophyll. In photosynthetic organisms, carotenoids exert an essential function in the photosynthetic apparatus.

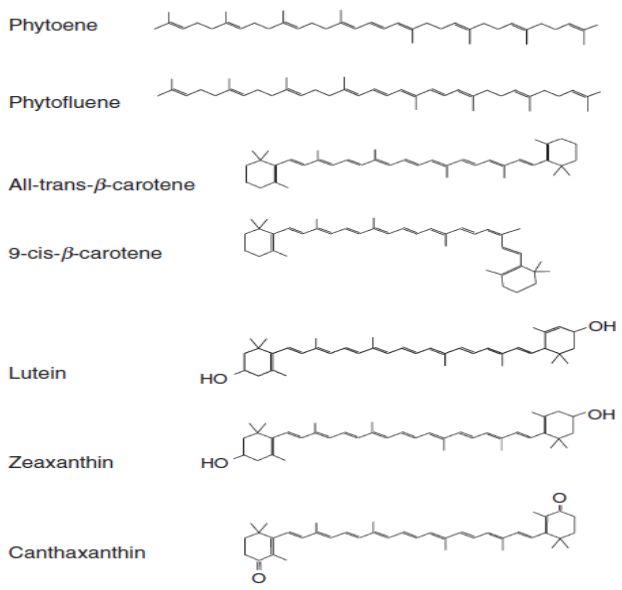

Figure 2.3: Structures of the biotechnologically most important carotenoids (Tafreshi and Shariati, 2009)

2.6 BETA-CAROTENE

As a member of carotenoids, beta-carotene is found in various structures as the configuration of each double-bond in beta-carotene can exist in trans or cis naturally (Ye et al., 2008). The all-trans, 9-cis, 13-cis and 15-cis stereo isomers of beta-carotene have been identified in natural sources (**Figure 2.4**). Beta-carotene accumulated within oil globules in the integranal thylakoid space of chloroplast and the 9-cis and all-trans isomer are in approximately equal amount, make up approximately 80% of the total (Gómez et al, 2003; Prieto et al., 2011). The beta-carotene is a terpenoid pigment that is highly valuable due to its nutritional benefit as a precursor of vitamin A for animals and for its antioxidant properties and it is also used for chemoprevention of some types of cancer (Gómez et al., 2003). Animals cannot synthesis carotenoids and therefore these pigments needed to be supplemented to them. Prieto et al. (2011) and Campo et al. (2007) stated that beta-carotene is a terpenoid pigment of increasing demand and a wide variety of market applications such as food colorants, as pro-vitamin A in food and animal feed, as an additive to cosmetics and multivitamin preparations and as a health food product under the antioxidant claim. Due to its capacity of accumulating large amounts of carotenoids in oil globules can be enhanced, *Dunaliella salina* is used worldwide as the main source of natural beta-carotene for industries (Lamers et al., 2008).